CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

January 25, 2010 — Date
David L. Parker — GINA SHIGMA

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Moritz Bünemann *et al.*

Serial No.: 10/538,985

Filed: August 18, 2006

For: MILLISECOND ACTIVATION SWITCH FOR SEVEN-TRANSMEMBRANE PROTEINS

Group Art Unit: 1646

Examiner: Pak, Michael D.

Atty. Dkt. No.: VOSS:008US

Confirmation No.: 2063

SUBMISSION OF SIGNED DECLARATION OF CARSTEN HOFFMANN

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This paper is submitted to replace the electronically signed Declaration of Carsten Hoffmann filed electronically via EFS Web on January 22, 2010, with an original copy bearing Carsten Hoffmann's signature.

It is believed that no fees are due in connection with this response. However, if any fees are due, consider this paragraph such a request and authorization to withdraw the appropriate fee under 37 C.F.R. §§ 1.16 to 1.21 from Fulbright & Jaworski L.L.P. Account No.: 50-1212/VOSS:008US.

The Examiner is invited to contact the undersigned at 512-536-3055 with any questions or comments.

Respectfully submitted,

Gina Simmons 45,104

For David L. Parker
Reg. No. 32,165
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201
(512) 536-4598 (facsimile)

Date: January 25, 2010

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FOR David L. Parker

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DECLARATION OF CARSTEN HOFFMANN

I, Carsten Hoffmann, hereby declare as follows:

1. I am a joint inventor of the subject matter disclosed and claimed in the referenced application. I have substantial expertise in the field of seven-transmembrane receptors and, in particular, G-protein-coupled receptors, as evidenced by my enclosed *curriculum vitae*. I am providing this declaration in order to present facts, demonstrating that the subject matter of the invention is fully enabled by the specification.

2. I understand that the subject matter of the invention is currently directed to G-protein coupled receptors comprising at least two detectable labels, wherein such labels are optionally positioned at or on the C-terminus, on the first intracellular loops or on the third intracellular loop. I further understand that the examiner has questioned the enablement of the application, particularly with respect to labels positioned in the first intracellular loop.

3. I understand that the examiner has stated that the specification fails to provide evidence supporting a broad enablement of the present invention. I strenuously disagree. For example, the present application convincingly demonstrates that prominent members of 7-transmembrane receptors, "namely the α_{2A}–adrenergic (neurotransmitter) receptor, the (adenosine) A2A-receptor and the parathyroid hormone (PTH hormone) receptors" (see, *e.g.*, page 7, bottom, of the specification), can indeed successfully be employed in accordance with the present invention (*e.g.* in the context of a "reliable, fast an easy measurement of the activation of such 7-transmembrane proteins"; see page 6 and 7 bridging paragraph, of the specification). This makes it clear that the present application provides evidence that 7-transmembrane receptors can generally be employed successfully in accordance with the teaching of the present application. Further evidence of enablement can be found in the fact that we have shown that a FRET-fluorophore does not negatively affect the receptor function, even if it is inserted into the first intracellular loop (see, *e.g.*, Fig. J, attached hereto). Thus, there is no basis for questioning that the introduction of FRET-fluorophores into other 7-transmembrane receptors will negatively effect their function, i.e. their function of being able to respond as a FRET sensor in a FRET assay according to the teaching of the invention.

4. Further with respect to Figure J, this figure shows the effects of an agonist on the FRET-response of the alpha2A receptor. Relative fluorescence intensity ratio was calculated from fluorescence emission measured at 480nm and at 530nm from cells expressing the receptor sensor and super-fused for the indicated period of time with the agonist nor-epinephrine (NE). The alpha2A receptor was labelled with CFP (donor fluorophore) at the C-terminus and FlAsH (acceptor fluorophore) within the first intracellular loop.

5. Figure J shows that receptor constructs can actually be generated also with labels inserted within the first intracellular loop, proving that functional receptor constructs in accordance with the entire breadth of claim 1 can be achieved. This is irrespective of the biological downstream function of the receptor, for example with respect to G-protein-coupling. Functional G-protein-coupling is not necessary to allow the receptor constructs to respond according to the invention in a receptor FRET assay. Therefore the Examiner's argument against loop 1 or loop 3 constructs at the end of page 5 turn to 6 disturbing G-protein-coupling is plainly invalid. We have shown in several publications (for example Vilardaga *et al.*, *Nature Biotechnology* 2003, Hoffmann *et al.*, *Nature Methods* 2005; both enclosed) that receptors with altered G-protein-coupling do still respond as FRET-sensors in accordance with the teaching of the present invention.

6. In order to further support the above line of argument that the examples provided in the present application can be generalized to basically all G-protein-coupled 7-transmembrane receptors, we herewith provide examples of further G-protein-coupled receptors which have successfully been used in accordance with the present invention. These further examples of G protein coupled receptors are (Figures attached):

(a) the human M1-muscarinic receptor (Figure A and B),

(b) the human M3-muscarinic receptor (Figure C and D),

(c) the human M5-muscarinic receptor (Figure E) and

(d) the human H1-histamine receptor (Figure G and H).

7. Each of the foregoing were labelled at the third intracellular loop and the C-terminus with the combination of FlAsH and CFP. Figures A, C, E and G display the ratio between the CFP and FlAsH fluorescence, whereas Figures B, D and H display the CFP and

FlAsH traces separately. Figure F displays the concentration-effect relationship for the M1, 3 and 5 muscarinic receptors which further underscores the utility of the recombinant G protein coupled receptor constructs of the present invention. All of the above exemplified further 7-transmembrane receptors have already been mentioned in the application as originally filed (see, e.g., page 40 of the specification, middle of the page) and belong to the group of G-protein-coupled receptors.

8. I submit that the foregoing provides strong evidence that the teaching of the present invention is broadly applicable to G protein coupled receptors in general. Furthermore, it is evident that Figure J provides supporting data that also the first intracellular loop can be labelled in order to create functional G protein coupled receptor constructs in accordance with the present invention.

9. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code.

January 22, 2010
Date

Carsten Hoffmann